



Institute Report No. 342

Acute Dermal Toxicity of
Triethyleneglycol Dinitrate (TEGDN) in Rabbits

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and
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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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SELECTED
FEB 27 1989
S H D

January 1989

Toxicology Series: 115

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

DISTRIBUTION STATEMENT A

Approved for public release;
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89 2 27 169

AD-A205 020

SECURITY CLASSIFICATION OF THIS PAGE


REPORT DOCUMENTATION PAGE				Form Approved OMB No 0704-0188	
1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION / AVAILABILITY OF REPORT		
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE			APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.		
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 342			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Division of Toxicology		6b. OFFICE SYMBOL (If applicable) SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION US Army Biomedical Research and Development Laboratory		
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800			7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5010		
8a. NAME OF FUNDING, SPONSORING ORGANIZATION US Army Medical Research & Development Command		8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER		
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012			10. SOURCE OF FUNDING NUMBERS		
			PROGRAM ELEMENT NO. 62720	PROJECT NO. A835	TASK NO. AB
					WORK UNIT ACCESSION NO. DA303913
11. TITLE (Include Security Classification) (U) Acute Dermal Toxicity of Triethyleneglycol Dinitrate in Rabbits					
12. PERSONAL AUTHOR(S) EW Morgan, and DW Korte, Jr.					
13a. TYPE OF REPORT Institute		13b. TIME COVERED FROM 15 NOV 84 TO 18 DEC 84		14. DATE OF REPORT (Year, Month, Day) January 1989	
15. PAGE COUNT 19					
16. SUPPLEMENTARY NOTATION Toxicology Series No. 115					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
			Acute Dermal Toxicity, Triethyleneglycol Dinitrate, TEGDN, Rabbit, Propellant, Munition. (JES)		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The acute dermal toxicity of triethyleneglycol dinitrate, TEGDN, was evaluated in five male and five female New Zealand White rabbits. Neat TEGDN (2 ml/kg) was applied topically to the clipped dorsal skin surface under a semi-occlusive wrap for 24 hours. No evidence was obtained of percutaneous absorption of quantities sufficient to produce systemic toxicity or death. Dermal signs could not be evaluated in two animals that began to molt during the study. Seven of the remaining eight rabbits exhibited very slight to slight erythema 1/2-hour after wrap removal and all but one had cleared by 72 hours. In this animal erythema persisted for 7 days after wrap removal. These data indicate that TEGDN does not produce systemic toxicity when administered by 24-hour topical application at a limit dose of 2 ml/kg. These data do suggest, however, that TEGDN has the potential to produce dermal irritation.					
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED		
22a. NAME OF RESPONSIBLE INDIVIDUAL EDWIN S. BEATRICE, COL, MC			22b. TELEPHONE (Include Area Code) (415) 561-3600		22c. OFFICE SYMBOL SGRD-ULZ

ABSTRACT

The acute dermal toxicity of triethyleneglycol dinitrate, TEGDN, was evaluated in five male and five female New Zealand White rabbits. Neat TEGDN (2 ml/kg) was applied topically to the clipped dorsal skin surface under a semi-occlusive wrap for 24 hours. No evidence was obtained of percutaneous absorption of quantities sufficient to produce systemic toxicity or death. Dermal signs could not be evaluated in two animals that began to molt during the study. Seven of the remaining eight rabbits exhibited very slight to slight erythema 1/2-hour after wrap removal and all but one had cleared by 72 hours. In this animal erythema persisted for 7 days after wrap removal. These data indicate that TEGDN does not produce systemic toxicity when administered by 24-hour topical application at a limit dose of 2 ml/kg. These data do suggest, however, that TEGDN has the potential to produce dermal irritation.

KEY WORDS: Acute Dermal Toxicity, Triethyleneglycol Dinitrate, TEGDN, Rabbit, Propellant, Munition

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DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
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Distribution/	
Availability Codes	
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A-1	



PREFACE

TYPE REPORT: Acute Dermal Toxicity GLP Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NO.: 84044

STUDY DIRECTOR: Don W. Korte Jr., PhD, LTC, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: Earl W. Morgan, DVM, MAJ, VC
Diplomate, American College of Veterinary
Preventive Medicine, American Board of Toxicology

PATHOLOGIST: G. Tracy Makovec, DVM, MAJ, VC
Diplomate, American College of Veterinary Pathologists

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Triethyleneglycol Dinitrate (TEGDN)

INCLUSIVE STUDY DATES: 15 November 1984 - 18 December 1984

OBJECTIVE:

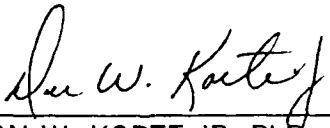
The objective of this study was to evaluate the acute dermal toxicity of TEGDN in male and female New Zealand White rabbits.

ACKNOWLEDGMENTS


LTC Larry D. Brown, DVM, SGT John R.G. Ryabik, BS, SP4 James J.Fischer, and SP4 Scott L. Schwebe assisted in conducting this research; SP4 Theresa L. Polk, SP4 Greg Rothhammer, Diane Arevalo, Charlotte L. Speckman, and Richard A. Spieler provided care for the animals; SGT Paul B. Simboli, BS, assisted in the chemical analysis; and Colleen S. Kamiyama, JoAnn Nishimoto, Dorothy Davis, and Julie Peacock provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY


We, the undersigned, declare that GLP Study 84044 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

 1 JAN 89

DON W. KORTE JR, PhD / DATE
LTC, MSC
Study Director

 2 JUL 87

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DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
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REPLY TO
ATTENTION OF

SGRD-ULZ-QA

3 January 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance Statement

1. This is to certify that the protocol for GLP Study 84044 was reviewed on 15 October 1984.
2. The institute report entitled "Acute Dermal Toxicity of Triethyleneglycol Dinitrate (TEGDN) in Rabbits," Toxicology Series 115, was audited on 20 December 1988.

Carolyn M. Lewis

CAROLYN M. LEWIS, MS

Diplomate, American Board of Toxicology
Chief, Quality Assurance

TABLE OF CONTENTS

Abstract	i
Preface	iii
Acknowledgments.....	iv
Signatures of Principal Scientists.....	v
Report of Quality Assurance Unit.....	vi
Table of Contents.....	vii
 INTRODUCTION.....	 1
Objective of Study.....	1
 MATERIALS.....	 1
Test Substance.....	1
Vehicle.....	2
Animal Data	2
Husbandry.....	2
 METHODS	 3
Acclimation/Group Assignment.....	3
Dose Levels.....	3
Test Procedures	3
Observations.....	4
Necropsy	4
Duration of Study.....	5
Changes/Deviations from Protocol.....	5
Raw Data and Final Report Storage.....	5
 RESULTS.....	 5
Mortality	5
Clinical Observations	5
Necropsy Findings.....	6
 DISCUSSION	 6
 CONCLUSION	 7
 REFERENCES	 8

TABLE OF CONTENTS (cont.)

APPENDICES	9
Appendix A. Chemical Data	10
Appendix B. Animal Data	12
Appendix C. Historical Listing of Study Events.....	13
Appendix D. Body Weight Data.....	14
Appendix E. Individual Dermal Signs	15
Appendix F. Pathology Report.....	17
OFFICIAL DISTRIBUTION LIST.....	19

Acute Dermal Toxicity of Triethyleneglycol Dinitrate (TEGDN) in Rabbits— Morgan and Korte

INTRODUCTION

The Department of Defense is considering the use of either diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in new propellant formulations. However, considerable gaps in the toxicology data of the compounds were identified during a review of their health effects (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL). Consequently, USABRDL has tasked the Division of Toxicology, Letterman Army Institute of Research (LAIR), to conduct an initial health effects evaluation of the proposed replacement nitrate esters. This initial evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP, includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the acute dermal toxicity of triethyleneglycol dinitrate (TEGDN) in male and female New Zealand White rabbits.

MATERIALS

Test Substance

Chemical Name: Triethyleneglycol Dinitrate (TEGDN)

Chemical Abstracts Service Registry No.: 111-22-8

LAIR Code Number: TA44

Morgan and Korte-2

Physical State: Liquid

Chemical Structure:



Molecular Formula: $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_7$

Source: Naval Ordnance Station
Indian Head, MD

Other test substance information is presented in Appendix A.

Vehicle

No vehicle was required as TEGDN is a liquid at room temperature.

Animal Data

Five male and five female young New Zealand White rabbits (Elkhorn Rabbitry, Watsonville, CA, USDA #93A74) from a shipment that arrived at LAIR on 15 November 1984 were assigned to the study. One rabbit (84F695) in the shipment was submitted for necropsy quality control on 16 November 1984. The 10 rabbits were identified individually by ear tattoos. The animal weights ranged from 2.2 to 2.6 kg on receipt and from 2.6 to 3.0 kg at dosing. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. No bedding was used in any of the cages. Water was provided *ad libitum* by continuous drip from a central line. The diet consisted of approximately 150 g per day of Purina Certified Rabbit Chow® No. 5322 (Ralston Purina Company, St Louis, MO). The animal room temperature was maintained in a range from 16.7-22.8°C with a relative humidity range from 24 to 68%. The photoperiod was 12 hours of light per day.

METHODS

This study was performed in accordance with LAIR Standard Operating Procedure OP-STX-30, "Acute Dermal Toxicity Study" (2) and Environmental Protection Agency guidelines (3).

Acclimation/Group Assignment

Study rabbits were quarantined by the Animal Resources Group (ARG), LAIR, for two weeks before being certified healthy by a staff veterinarian. After being certified healthy, the rabbits were transferred to the Toxicology Suite for the remainder of the study.

Randomization for group assignment was unnecessary as there was only one dose level for each sex.

Dose Levels

A "limit test" was conducted in which 5 male and 5 female rabbits were assigned to a test group receiving 2.0 ml/kg of TEGDN applied topically to the dorsum (skin over back). According to body weight, 4.0 to 4.5 ml of neat TEGDN was applied to each rabbit.

Test Procedures

The application sites on the dorsal and lateral sections of the animals (surface area approximately 300 cm²) were close-clipped with electric clippers (Oster® Model A5, Size 40 blade, Sunbeam Corp, Milwaukee, WI) 24 hours before applying the test compound. The animals were weighed, and the volume of compound required to provide the 2.0 ml/kg limit dose was measured. This quantity of the test compound was evenly distributed over the surface of an 7 x 7 in. piece of gauze dressing (Curity Cover Sponges, Kendall Co, Hospital Products, Boston, MA) which was then taped to the animal's back with hypoallergenic tape (Durapore® Surgical Tape, 3M Corp, St Paul, MN). The trunk of the animal was then wrapped with Vetrap® bandaging tape (Animal Care Products, 3M Corp, St Paul, MN) to hold the compound in place and prevent the animal from ingesting the compound. The Vetrap® was

anchored in place cranially and caudally by strips of Conform® elastic tape (Kendall Co, Hospital Products, Boston, MA). The patch and wrappings were left in place for 24 hours. No restraint of the animals was used except during the wrapping procedure. When the wrappings and patch were removed the exposed area was gently wiped with a piece of saline-moistened gauze to remove any remaining test compound.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (1) animals were observed undisturbed in their cages, (2) animals were removed from their cages and given a physical examination, and (3) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Observations were recorded daily for the remainder of the two-week test period. A second "walk through" observation was performed each day, with only significant observations recorded. The exposed area was examined 1/2, 24, 48 and 72 hours after patch removal, and all lesions were noted and graded as described below. Animals were weighed weekly during the study test period.

During evaluation of the exposure site, area and intensity of each dermal reaction were graded. Grading was performed according to a scale which included five categories to describe area and severity. Area categories were 0 - 5%, > 5 - 10%, > 10 - 25%, > 25 - 50% and > 50%; severity was defined as very slight, slight, moderate, well-defined, and severe. Dermal reactions which were graded included erythema and molting.

Necropsy

All study animals were submitted for necropsy. Those which survived the 14-day study period were necropsied immediately after being given an overdose of sodium pentobarbital and sacrificed by exsanguination from severed axillary vessels. Skin was taken from the exposed area and examined microscopically.

Duration of Study

The study period was 14 days and was preceded by a 19-day quarantine. Historical study events are listed in Appendix C.

Changes/Deviations from Protocol

During the week before dosing the relative humidity increased throughout the week. During 30 Nov - 2 Dec 84 the relative humidity ranged from 80 to 92%. The problem with the humidity control was corrected on the day before dosing. To facilitate application of the test compound, the test sites were clipped again as required on 4 Dec 84 (day of dosing). These deviations had no apparent effect on the study.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Mortality

Twenty-four hour dermal exposure to TEGDN at a limit dose of 2.0 ml/kg produced no mortality in the 10 rabbits evaluated in the study.

Clinical Observations

During the course of the study, observations were split into two major categories: systemic (general health of the animal) and dermal.

Systemic: Three rabbits had diarrhea as indicated by presence of fecal material on perianal fur or on the cage floor; rabbit 84F693 at 3 hours after dosing, rabbit 84F707 on days 1 and 2 after dosing, and rabbit 84F689 on days 6 and 7 after dosing. Rabbit 84F689 also appeared anorexic on days 6 and 7 after dosing. None of the clinical systemic signs observed were interpreted as signs of toxicity attributable to the test compound. In addition,

the rabbits gained weight, as expected for young animals, during quarantine and after administration of TEGDN (Appendix D).

Dermal: Skin irritation signs are presented in Appendix E. Rabbits 84F693 and 84F705 exhibited molting during the study which precluded accurate evaluation of skin irritation in these animals. Of the remaining 8 animals, 7 presented with erythema 1/2-hour after patch removal. By 72 hours this erythema had disappeared in all but one of these animals. In rabbit 84F690 slight erythema persisted through day 7 after dosing.

Necropsy Findings

There were no gross or microscopic findings in these rabbits at necropsy, following the 2-week observation period, that could be attributed to dermal exposure to TEGDN at the 2 ml/kg dose level. A copy of the complete Pathology Report appears in Appendix F.

DISCUSSION

Acute dermal toxicity testing is designed to evaluate both systemic toxicity due to percutaneous absorption of the test material and local toxicity from its contact with the skin. From these observations it can be determined whether absorption of the test material across the skin is sufficient to produce systemic effects or lethality. In the present study, triethyleneglycol dinitrate produced slight local dermal reactions with no evidence of systemic effects.

All of the animals exposed to a limit dose of 2.0 ml/kg TEGDN survived to the end of the test. None of these test animals exhibited any clinical signs suggestive of a systemic action by TEGDN. This lack of dermal systemic toxicity is in marked contrast with the results of the acute oral toxicity studies. In the acute oral toxicity studies the median lethal dose (MLD) was determined to be 1.8 to 2.1 g/kg for ICR mice and 1.1 to 1.3 g/kg for Sprague-Dawley rats (4). Therefore, it is concluded that dermal exposure to TEGDN, at 2.0 ml/kg, either does not result in sufficient percutaneous

absorption to produce systemic toxicity or is not a systemic toxin at doses tested in the rabbit. The dermal median lethal dose of TEGDN, as indicated by this study, is above the limit value of 2.0 ml/kg.

Local dermal toxicity was observed at the site of exposure. As summarized in Appendix D, very slight to slight erythema was present in 7 of 8 nonmolting animals 1/2-hour after the removal of test compound wrappings. This is consistent with the dermal irritation study results in which TEGDN was classified as a mild irritant (4).

CONCLUSION

A limit dose of 2.0 ml/kg triethyleneglycol dinitrate was not lethal to rabbits nor did it produce significant systemic effects following dermal exposure for 24 hours; however, it did exhibit the potential for slight dermal irritation.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846.
2. Acute dermal toxicity study. LAIR Standard Operating Procedure OP-STX-30, Presidio of San Francisco, CA: Letterman Army Institute of Research, 18 May 1984.
3. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, dermal toxicity. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.
4. Brown LD, Korte DW, Jr. Primary dermal irritation potential of triethyleneglycol dinitrate (TEGDN) in rabbits. Toxicology Series 140. Presidio of San Francisco, CA: Letterman Army Institute of Research, 1989; Institute Report No. 339.

Appendix A. Chemical Data	10
Appendix B. Animal Data	12
Appendix C. Historical Listing of Study Events.....	13
Appendix D. Body Weight Data.....	14
Appendix E. Individual Dermal Signs	15
Appendix F. Pathology Report	17

Morgan and Korte-10

Appendix A: CHEMICAL DATA

Chemical Name: Ethanol, 2,2'-[1,2-ethanediylbis(oxy)] bis-, dinitrate

Alternate Chemical Names: Triethyleneglycol dinitrate, NOSET-A

Chemical Abstracts Service Registry No.: 111-22-8

LAIR Code Number: TA44

Chemical Structure:



Molecular Formula: C₆H₁₂N₂O₈

Molecular Weight: 240

Physical State: Yellow oil

Density: (g/cm³): 1.32*

Manufacturer: Naval Ordnance Station
Indian Head, MD

Lot No.: 130-84

* Holleman JW, Ross RH, Carroll JW. Problems definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983; Oak Ridge National Laboratory Publication No.EIS-202. p17.

APPENDIX A (cont.): CHEMICAL DATA

Analytical data: The compound chromatographed as a single peak (retention time 5.8 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% methanol; flow rate 0.9 ml/min, detection wavelength, 215 nm.[†] No impurities were detectable by NMR.[‡] NMR (80 MHz, CDC1₃): 3.65 (S, 4H, -CH₂-O-CH₂CH₂-O-CH₂-), 3.72-3.84 (Complex multiplet, 4H, terminal methylene groups). IR (KBr): 2900, 1630, 1280, 1130, 1030, 910, 860 cm.[§]

Stability: The compound was received as a 10% solution in ethanol. Periodic analysis of this solution by HPLC has shown no evidence of decomposition to date (4 months).[†] NMR analysis demonstrated that the neat compound is stable for at least 1 month.[‡]

[†] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p26-30, 42-43. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[‡] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p63. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[§] Ibid. p64.

Appendix B: ANIMAL DATA

Species: *Oryctolagus cuniculus*

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male and Female

Age: DOB: Females 18 Aug 84
Males 24 Aug 84

Animals in each group: 5 males and 5 females

Condition of animals at start of study: Normal

Body weight range at dosing: 2.62 - 2.97 kg

Identification procedures:

Ear tattoo procedure (SOP OP-ARG-1), tattoo numbers 84F689-84F693, females, and 84F704-84F708, males (inclusive).

Pretest conditioning:

1. Quarantine from 15 November - 3 December 1984
2. Animals were close-clipped and examined 24 hours before dosing

Justification:

The laboratory rabbit is a proven mammalian model for dermal toxicity studies because of its size, ease of restraint, and skin permeability.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

<u>DATE</u>	<u>EVENT</u>
15 Nov 84	Animals arrived at LAIR. They were held for a two-week quarantine period.
15 Nov - 4 Dec 84	Animals were observed daily for illness.
16 Nov 84	Animals were tattooed.
16,23 Nov 84	Animals weighed by Animal Resources Group (ARG) personnel.
28 Nov 84	Animals were removed from quarantine after being certified as healthy by an ARG veterinarian.
28 Nov - 4 Dec 84	Animals were acclimated to the GLP Suite.
29 Nov, 4,11, 18 Dec 84	Animals were weighed.
3 Dec 84	Animals were close-clipped.
4 Dec 84	Animals were reclipped as needed and dosed. Observations and clinical signs were recorded three times.
5-17 Dec 84	Animals were observed twice daily for two weeks.
18 Dec 84	Feed was removed during the morning observation. Animals were submitted to the Necropsy Suite.

Appendix D: BODY WEIGHT DATA

<u>Animal Number</u>	<u>Day</u>					
	<u>01</u>	<u>08</u>	<u>014</u>	<u>0</u>	<u>7</u>	<u>14</u>
<u>Females</u>						
84F689	2450	2655	2629	2716	2808	2919
84F690	2445*	2570	2680	2786	2971	3107
84F691	2475	2655	2600	2845	3163	3224
84F692	2180	2675	2632	2780	3015	3095
84F693	2500	2705	2545	2746	2889	2873
Mean	2410.0	2652.0	2617.2	2774.6	2969.2	3043.6
Standard Deviation	130.4	50.2	49.5	48.4	134.2	144.8
<u>Males</u>						
84F704	2420	2580	2521	2761	3011	3005
84F705	2210	2420	2420	2625	2806	2981
84F706	2515	2630	2620	2760	2863	2863
84F707	2225	2260	2444	2623	2898	2881
84F708	2630	2730	2878	2973	3090	3234
Mean	2400.0	2524.0	2576.6	2748.4	2933.6	2992.8
Standard Deviation	182.5	185.3	185.7	142.9	115.1	148.2

* Weights are given in grams.

Appendix E: INDIVIDUAL DERMAL SIGNS

Table E-1: Males

<u>Animal Number</u>	<u>Dermal Signs</u>	<u>Duration of Dermal Signs(Days)</u>	<u>Severity*</u>	<u>Area†</u>
84F704	Erythema	1-2	A	5
84F705	Molting	1-10	C	3-2
84F706	Erythema	1-2	A	5
84F707	Erythema	1-2	A	5
84F708	Erythema	1	A	5

* Severity Scores

A = Very Slight
 B = Slight
 C = Moderate
 D = Well-defined
 E = Severe

† Pertains to percent of exposed area exhibiting signs of dermal irritation.
 This value is determined by visual approximation.

1 = 5%
 2 = > 5 to 10%
 3 = >10 to 25%
 4 = >25 to 50%
 5 = >50%

Appendix E (cont): INDIVIDUAL DERMAL SIGNS**Table E-2: Females**

<u>Animal Number</u>	<u>Dermal Signs</u>	<u>Duration of Dermal Signs(Days)</u>	<u>Severity*</u>	<u>Area†</u>
84F689	Erythema	1,3	A-B	5-1
84F690	Erythema	1-7	A	5-1
84F691	Erythema	1	B	5
84F692	None	N/A	N/A	N/A
84F693	Molting	1-10	B-E	3

* Severity Scores

A = Very Slight
 B = Slight
 C = Moderate
 D = Well-defined
 E = Severe

† Pertains to percent of exposed area exhibiting signs of dermal irritation.
 This value is determined by visual approximation.

1 = 5%
 2 = > 5 to 10%
 3 = >10 to 25%
 4 = >25 to 50%
 5 = >50%

Appendix F: PATHOLOGY REPORT

Pathology Report
GLP Study 84044
Acute Dermal Toxicity (LD₅₀) Test

Substance: Triethyleneglycol Dinitrate (TEGDN) Species: Rabbit, New Zealand White.

Age: Approximately 4 months old, 5 male, 5 female.

History: See LAIR SOP-OP-STX-30. "Acute Dermal Toxicity". All animals were killed.

Gross Necropsy Findings:

36482 - 84F689	Female - Uterus - hydrometra, left uterine horn.
36483 - 84F690	Female - No lesions.
36484 - 84F691	Female - Ears - otitis media, purulent, bilateral.
36485 - 84F692	Female - Liver - four white foci, 2-4mm in diameter Ears - otitis media, purulent, bilateral
36486 - 84F693	Female - Skin - diffuse red mottling over spine; Ears - otitis media, purulent, bilateral.
36487 - 84F704	Male - No lesions.
36488 - 84F705	Male - No lesions.
36489 - 84F706	Male - Cecum - contained parasites.
36490 - 84F707	Male - Ears - otitis media, purulent, bilateral.
36491 - 84F708	Male - Liver - multiple 1mm white foci; Cecum - contained parasites.

Microscopic Findings: Skin sections control and treated.

36482-1 (control): Not Remarkable (NR).

36482-2 (treated): NR.

36483-1: Toward the end of one section there are a few small blood vessels in the superficial layer of dermis that are surrounded by small infiltrates of heterophils.

Dx: Perivascular heterophil infiltrates, focal, minimal, dermis, skin.

36483-2: NR.

Appendix F (cont): PATHOLOGY REPORT

Pathology Report
GLP Study 84044

36484-1: In both sections there is a small focal infiltrate of heterophils and macrophages in the superficial dermis.

Dx: Dermatitis, heterophilic and histiocytic acute, focal, minimal, dermis, skin.

36484-2: NR.

36485-1: NR.

36485-2: NR.

34686-1: NR.

34686-2: NR.

34687-1: NR.

34687-2: NR.

34688-1: NR.

34688-2: NR.

34689-1: NR.

34689-2: NR.

34690-1: NR.

34690-2: NR.

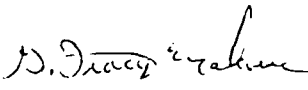
34691-1: NR.

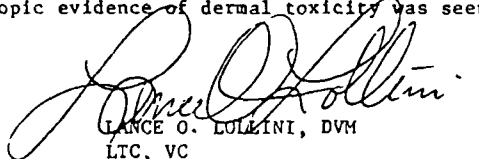
34691-2: There are microfocal infiltrates of a few macrophages and lymphocytes in the superficial layer of the dermis.

34691-3: Liver Dx: Coagulative necrosis, acute, multifocal, mild, with minimal infiltrates of heterophils and macrophages, liver.

Dx: Pericholangitis, lymphocytic and histiocytic, chronic, multifocal, marked with intraductal coccidia, liver.

Comments: The gross lesions were considered incidental findings and not related to the treatment. No microscopic evidence of dermal toxicity was seen.


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